Spatially offset Raman spectroscopy (SORS) for the analysis and detection of packaged pharmaceuticals and concealed drugs

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Abstract

Spatially offset Raman spectroscopy (SORS) is a powerful new technique for the non-invasive detection and identification of concealed substances and drugs. Here, we demonstrate the SORS technique in several scenarios that are relevant to customs screening, postal screening, drug detection and forensics applications. The examples include analysis of a multi-layered postal package to identify a concealed substance; identification of an antibiotic capsule inside its plastic blister pack; analysis of an envelope containing a powder; and identification of a drug dissolved in a clear solvent, contained in a non-transparent plastic bottle. As well as providing practical examples of SORS, the results highlight several considerations regarding the use of SORS in the field, including the advantages of different analysis geometries and the ability to tailor instrument parameters and optics to suit different types of packages and samples. We also discuss the features and benefits of SORS in relation to existing Raman techniques, including confocal microscopy, wide area illumination and the conventional backscattered Raman spectroscopy. The results will contribute to the recognition of SORS as a promising method for the rapid, chemically-specific analysis and detection of drugs and pharmaceuticals.

Keywords

Spatially offset Raman spectroscopy, drug detection, pharmaceutical analysis, laser, diffuse light, turbid media.

Introduction

The need for new techniques to rapidly screen packages, mail, bottles and other items has been underscored by the recent escalated activities of organized crime and terrorist groups. It is vital to find sensitive and selective techniques for identifying concealed substances, including drug mixtures, chemical and biological warfare agents, explosives and toxic substances without opening a suspected package, in order to minimize possible harmful exposure [1].

There is also growing concern regarding the counterfeiting of pharmaceutical tablets and medications [2]. Fake or substandard drugs may contain too much, too little, or the wrong active ingredient and/or toxic ingredients [3]. In wealthy countries, counterfeiting often involves expensive hormones, steroids, anti-cancer medicines and so-called 'lifestyle' drugs [4]. However in developing countries, such as in Africa, counterfeit medicines are commonly encountered in the treatment of life-threatening conditions such as malaria, tuberculosis and HIV/AIDS [2-4].

Different techniques have been proposed for the detection and identification of drugs and hazardous substances. These include HPLC/MS, GC/MS, ion mobility spectroscopy, molecularly

imprinted polymers, various optical methods and several others [1, 2]. Many of these techniques are invasive and require a certain level of sampling to introduce the suspected substance to the instrument. Further, some are inherently laboratory-based techniques that are not readily adaptable to field investigations.

In the face of these emerging challenges, a new development has been the advent of spatially offset Raman spectroscopy (SORS) [5]. This technique is based on Raman scattering, a phenomenon discovered by C.V. Raman in 1928 [6]. Raman scattering is where photons of incoming light are inelastically scattered from the molecular vibrations of the sample, which causes the energy (wavelength/frequency) of the photon to be altered [7]. For many decades, Raman scattering has been the basis of a powerful spectroscopic technique. Since the particular energy losses incurred by the incoming photons are related to the structure of the molecule, the Raman spectrum measured from the compound provides a unique 'fingerprint' that reflects its chemical constituency [7]. It is therefore apparent that, in the context of drug identification and analysis, Raman spectroscopy is an extremely powerful technique because it offers detection based on the unique spectrum of the compound [1, 7, 8].

In its conventional mode, Raman spectroscopy involves introducing a laser beam onto the sample to produce a small illuminated spot on its surface [7]. A lens positioned adjacent to the sample then collects the light emanating from this illuminated spot; that is, the focal point of this lens is precisely aligned to the illuminated spot. The sampling depth from which the Raman-scattered photons are collected is defined by the focal volume of the lens. Tightly focused optics (such as microscope objectives) are generally used, and therefore the collection volumes typically extend no more than a few hundred microns deep into the sample. Thus, the conventional Raman approach is naturally biased towards collecting photons that originate from, or at very shallow depths beneath, the surface of the sample. Since the illumination and collection zones coincide, any fluorescence generated by the sample will also be collected. In some cases, the fluorescence may be sufficient to 'swamp' the detector and thus degrade, or preclude entirely, the detection of the relatively much weaker Raman photons [7, 9].

The SORS innovation has been to adapt this long-standing spectroscopic technique into a mode that allows the retrieval of *pure, subsurface Raman spectra,* emanating from much deeper layers than conventional Raman can analyze [5]. SORS has been demonstrated for retrieving Raman spectra of samples that are situated behind opaque layers of non-absorbing materials such as polymers, paper, glass, powders, turbid materials and even biological media [9].

SORS involves the acquisition of two Raman spectra, followed by a scaled subtraction which then yields the subsurface Raman spectrum. The first spectrum is obtained from the container surface using coincident zones of collection and illumination, and is thus akin to a conventional Raman measurement. In the examples presented here, this first spectrum will

emphasize the Raman signal emitted by the container wall (although a contribution from the concealed substances may also be apparent). The second measurement is also taken at the surface of the container, but at a position spatially offset from the illumination zone, and this measurement is effective at capturing subsurface photons [5].

The original discovery of SORS was by Matousek and colleagues in 2005 using offset spot illumination (Figure 1a; "SORS") [5]. In brief, the SORS principle is as follows. Laser light incident on the sample penetrates (with exponentially decaying intensity) into the diffusely scattering sample, with some photons reaching depths of up to a few centimeters. As the photons propagate through such a turbid medium, their original straight trajectory is rapidly converted to a range of sideways scatters, which eventually completely randomize the path of the photon [10]. Provided the upper layer (container wall) is not too thick, some of the photons will reach the subsurface compound, i.e. the concealed substance inside the container. By the time this has occurred, however, many of these photons will have been displaced sideways from their original path of entrance into the sample; that is, the photons will have spread out to create a larger volume of diffuse (albeit very weak) light within the sample [5, 10]. Those photons that are randomly scattered back towards the container wall and reemerge will also, on average, have been scattered further sideways on their way back to the surface. Thus, on average, the deeper the photon has travelled into the sample, the further away from the original point of entry (i.e. the illumination spot) that it will reemerge from the container surface [5]. Collecting photons from greater spatial offsets will tend to capture Raman photons that have originated from greater depths within the sample. (Notably, however, deeper-traversing photons will also be more strongly attenuated, which effectively limits the extent of spatial offset from which photons can still be recovered, as determined by the lower limit of detection of the spectroscopic equipment).

Most of these scattering events will be Rayleigh (elastic) scattering, with the light remaining at the original laser wavelength. Nonetheless, some of these events will involve Raman scattering. Consequently, although the predominance of emerging photons will be Rayleigh photons – and therefore useless for spectroscopic analysis – there will also be a contribution of Raman photons. Collecting these photons at an appropriate offset will thereby bias the collection towards the Raman signal of the underlying layers. In comparison, the Raman photons originating from the surface decay very rapidly with increasing offset from the illumination point, since they have had much less opportunity to travel sideways by scattering, than those emerging from underlying layers [5]. (Note that the offset spectrum may still include contributions of Raman photons and fluorescence from the container wall).

The subsurface Raman spectrum is obtained by a scaled subtraction of the two spectra: the spatially offset spectrum (container wall plus contents) minus the spot spectrum

(predominantly a container spectrum), with appropriate scaling, will yield a pure spectrum of the concealed compound [5]. This will be illustrated, step-wise, in the first example below of powdered phenylephrine hydrochloride (a common decongestant) within an opaque white plastic bottle. The other key feature of SORS that facilitates retrieval of the weak subsurface signal is that, because the collection and illumination volumes are spatially separated, the amount of fluorescence gathered by the collection optics is greatly reduced [5].

The *inverse SORS* geometry was subsequently introduced [11, 12], based on using ring-shaped rather than point illumination for the spatial offset, and this is the geometry that will be used here (Figure 1a; "Inverse SORS"). It overcomes potential spectral distortions inherent in SORS instrumentation (discussed in [11]) and also allows the excitation light to be spread over a larger area, thus providing for higher laser powers and reduced acquisition times. The spatial offset is given by the radius of the ring; measuring the signal emanating from the center of the ring allows for collection of Raman photons originating from the subsurface layers [11, 12].

It is important to note that there are other valuable approaches for probing diffuse media including terahertz, NIR and MIR spectroscopy, as well as x-rays and ultrasound. A key advantage of Raman analysis over some of these techniques, however, is that it yields chemically specific spectra [7, 9]. It is also possible to analyze mixtures and determine their composition using multivariate statistical analysis from the overall Raman spectrum of the mixture, by comparison against a stored library of compounds [8]. Raman spectroscopy is also suited to field deployment in the form of a handheld portable device [1].

A major factor that will assist the widespread uptake of SORS will be an increased understanding of its implementation in various real-world scenarios. In this paper, we aim to contribute some examples and considerations for using SORS in different applications relevant to forensics. We provide case studies of the use of SORS for interrogating a range of containers and samples, including an antibiotic in a blister pack, a drug dissolved inside a clear solution (as a model for transportation of illicit drugs in alcoholic beverages), a powdered drug in an opaque plastic container, a multi-layered padded post-bag containing a powdered agent, and a paper envelope containing a thin layer of powdered substance. We also discuss some characteristics, benefits and limitations of the SORS technique applicable to its use in the field.

Material and methods

The experimental setup is shown in Figure 1b. The light source is a 450 mW temperature- and current-stabilized 785 nm diode laser (BRM-785; BWTek), which is spectrally filtered (LD01-785/10-25; Semrock) to remove residual laser emission. To perform SORS, an optical element is required that offers switchable modes of ring-shaped and spot illumination. This was achieved with an axicon lens (Del Mar), which has a conical face that refracts the emerging laser

beam [11]. At the effective focal length of the axicon, a spot is produced that is approximately equal in diameter to the laser beam (~ 4 mm). In our setup, the axicon is mounted on a 250 mm-length cage-rail system (ThorLabs Inc.) so that the axicon can be slid away from the sample. Beyond the effective focal length, the light emanating from the axicon lens gradually diverges, producing a ring of light that increases in diameter with increasing distance from the axicon. Thus, as the distance between the sample and axicon lens is increased (by means of sliding the lens along the rails), a ring of larger diameter is formed on the sample. The ring illumination used here had an outer diameter of approximately 16 mm. To conduct SORS measurements, spectra were separately captured for spot and ring-shaped illumination and then a scaled subtraction was performed (ring – spot) with software.

For all of the spectra, the Raman photons were captured in a backscattering geometry (Figure 1b) using a 50 mm diameter biconvex lens with a focal length of 60 mm; i.e., the stand-off distance was 60 mm in the below experiments. After collection, the light traverses a 50 mm diameter notch filter that suppresses the 785 nm elastically scattered (Rayleigh) light. A second convex lens (60 mm focal length) focuses the light onto the optic fiber bundle (19 fibers bundled together, each with 200 μm core diameter) (Princeton Instruments). Another notch filter and an edge (long-pass) filter are situated in front of the fibers to suppress residual 785 nm light.

At the entrance of the spectrograph (SP2360; Princeton Instruments), the 19 fibers are stacked in a vertical strip (~ 4 mm in height) that is aligned to the 200 μm-wide entrance slit. Spectra are measured using a high-sensitivity camera (PIXIS 256; Princeton Instruments) in which the 256 x 1024-pixel CCD is thermoelectrically cooled to –70 °C to suppress noise. All 256 CCD strips are vertically binned into a single spectrum and acquired on the PC (WinSpec, Princeton Instruments). Spectra were corrected using a background spectrum captured when the laser was off. Tests were conducted in darkness and spectra were not corrected for variations in CCD pixel sensitivities.

The target compounds used were: phenylephrine hydrochloride powder (P6126; Sigma); acetaminophen (paracetamol) powder (A7085; Sigma); barium sulphate (> 99%; Sigma) and an antibiotic capsule containing amoxicillin and flucloxacillin (Flumox® 500 mg, EIPI Co.). AR-grade methanol was used in the tests involving dissolved acetaminophen. Local regulations precluded us acquiring and testing illicit drugs (e.g. cocaine, heroin, etc.). However, a successful demonstration of the technique for the above substances would indicate SORS is viable for most other drugs and compounds, since they will also be Raman-active and have unique spectra that allow their identification.

Reference spectra for each of the substances were obtained by screening unconcealed samples with the device. In practice, unknown substances would be identified by matching the obtained SORS spectrum with the unique Raman spectrum from a library of target substances.

Results and Discussion

Results from each sample and package are presented below. To show the quality of the collected raw data, no curve smoothing or chemometric alterations have been undertaken on the following spectra.

Phenylephrine inside a plastic container

The SORS probe was used to analyze powdered phenylephrine hydrochloride contained in an opaque white plastic jar, which is similar to those used to package pharmaceutical tablets (1 mm wall thickness; high-density polyethylene (HDPE)). Raman spectra were acquired while exposing the container wall to spot and ring-shaped illumination. The exposure time for each spectrum was 10 seconds (10 × 1 second accumulations). The Raman bands from the container (marked with an asterisk) and concealed phenylephrine are visible in both spectra (Figure 2; top two axes). Notably, however, the ring measurement has suppressed the container peaks relative to the phenylephrine peaks.

To perform a scaled subtraction, the spectra were first normalized so that the container peaks overlapped (Figure 2; middle axes). Following subtraction, the container peaks were removed and the resultant spectrum closely matched the reference spectrum for phenylephrine hydrochloride, thus revealing the identity of the concealed drug (Figure 2).

This result demonstrates the capability of SORS to retrieve pure Raman spectra of concealed compounds through diffusely scattering polymer containers. Of particular note is that SORS does not require prior knowledge of the container material or a measurement of its Raman spectrum to be performed [5]. There are a range of examples in the literature where SORS has been successfully used to interrogate various diffusely scattering plastic containers [4, 9], and in many cases the spectral processing was automatically performed by software and without specialist input [9].

Antibiotic capsule in blister pack

Measurements of capsules retained in their blister packaging were reported as early as the 1980s using confocal Raman spectroscopic techniques. Such confocal procedures require precise placement of the tablet at the focal plane of the instrument's optics to provide discrimination of Raman signals captured from the capsule contents. In the case of colored capsule shells or plastic packaging, it may be difficult to obtain clear Raman spectra above background levels of fluorescence or Raman emission [4].

Matousek and colleagues reported successful use of the SORS technique to retrieve Raman spectra from a range of off-the-shelf cold and flu tablets and capsules [4, 13]. The researchers

used the non-inverse SORS technique in which a 1 mm-diameter spot (from an 827 nm laser source) was incident upon the capsule and the spatially-offset spectrum was collected several millimeters away on the capsule [4]. Here we report successful interrogation of an antibiotic tablet (amoxicillin and flucloxacillin) with a darkly colored shell, retained inside its blister pack, using the inverse SORS geometry.

The unopened blister pack was positioned in front of the inverse SORS instrument and spectra were acquired for spot and ring illumination (10 × 1 second accumulations). Because of the large ring diameter, portions of the light fell either side of the capsule, although this evidently did not degrade the results. The spot spectrum contained a large fluorescence background from the outer layers (Figure 3). The ring spectrum contained perceptible Raman bands, as well as fluorescence. The spot spectrum was subtracted from the ring spectrum so that the fluorescence was removed, and the resultant spectrum closely matched the reference Raman spectrum of the capsule contents (Figure 3).

Analysis of tablets by non-destructive means is important in many applications [3, 7, 9]. In pharmaceutical manufacturing, it may be necessary to assess the bulk content of tablets to ensure consistency in tablet composition and to screen for impurities. Raman spectroscopy is a proven tool for the analysis of pharmaceutical products [7], although in the conventional backscattered geometry it generally cannot reach sufficient depths to probe the overall contents of tablets [14, 15]. Matousek and Parker have also demonstrated the use of a transmission geometry for this purpose [14]. Transmission Raman may be treated as an extreme case of SORS, in that the excitation and collection occur on opposite sides of the sample [14]. They tested a paracetamol tablet with a thin impurity layer of *trans*-stilbene powder that was positioned on the front and rear faces of the tablet (the two extreme positions of possible impurity depth). The impurity was detectable with a similar level of sensitivity in both cases. This illustrates that the transmission Raman geometry is unaffected by the position of the impurity within the tablet, since the entire tablet is probed by the laser [14]. Some applications may require screening of capsules or tablets with coatings. The transmission geometry can also collect excellent Raman spectra of these items, because it involves a spatial offset between the excitation and collection zones which consequently suppresses the collection of fluorescence and Raman signals emanating from the exterior layers [13]. If necessary, a spot spectrum (i.e., of the interfering outer layers) could be obtained and subtracted, similar to the SORS approach. Since transmission and spatially-offset techniques generally use wider-area beams than conventional backscattering Raman (to probe larger sample volumes), this enables the use of substantially higher laser powers. This provides for short analysis times on the order of seconds or less [13, 14]. Such features make these techniques of great value for the analysis of pharmaceutical products, particularly for loose unpackaged tablets during production [9].

In the case of packaged tablets, it may be more desirable to perform *in situ* analysis using SORS. The two most common types of pharmaceutical packaging are blister packs and opaque plastic jars. The foil backing of blister packs would be impenetrable to NIR light and thus it may be difficult to orient the collection and illumination optics on opposite sides of the probed capsule to undertake transmission measurements. The inverse SORS geometry, such as demonstrated here, may be more practical. The dimensions of most plastic jars are too large to allow transmission of NIR light. However, SORS can retrieve Raman spectra from depths up to several centimeters inside plastic jars; since excitation and collection are located on the same side of the jar, SORS is compatible with jars of varying dimensions. Furthermore, Eliasson and Matousek [4] have demonstrated acquisition of Raman signals from capsules and coated tablets inside plastic jars, with effective suppression of both the container signal as well as from the outer layers on individual tablets inside using the SORS approach. SORS may be a useful tool for combating the increasing prevalence of counterfeit and fake drugs [3]. It is becoming increasingly important to verify drug content throughout the entire supply chain [4].

These results and the foregoing studies [3, 4, 9, 13, 14] illustrate the practical utility of spatially offset Raman techniques for the analysis of pharmaceutical tablets and capsules in a range of applications. Different geometries (SORS or transmission Raman) should be adopted to accommodate for particular types of tablet packaging, container designs and container materials. These approaches allow for the non-invasive *in situ* analysis of packaged products and rapid non-destructive screening of loose tablets [3, 13, 14].

Dissolved acetaminophen in methanol inside a plastic bottle

There is increasing concern regarding the transportation of illicit drugs dissolved in alcoholic beverages. It is relatively easy to disguise a large quantity of a soluble drug inside a standard 700 mL liquor bottle, e.g. ~ 300 g of cocaine in a rum bottle [16]. It then becomes a formidable task for customs officials to screen the large numbers of alcoholic beverages that are transported as cargo or carry-on luggage. Without spectroscopic techniques, for example, it may only be possible to detect the presence of the drug by pouring the beverage and judging that its viscosity has increased due to the presence of a dissolved solute.

Several hand-held Raman devices are demonstrated to provide effective detection through transparent bottles including darkly colored wine bottles. Dark glass bottles are notoriously highly fluorescing and are difficult targets for Raman analysis, especially using conventional geometries [17]; nevertheless these instruments can generally retrieve a Raman spectrum above the fluorescence background. Eliasson et al. [16] used a 'displaced Raman' technique, building on SORS, to detect cocaine in rum to an estimated detection limit of ~ 9 g per 0.7 L. The displaced Raman technique introduces the laser beam into the bottle at an angle so that its path

intersects with the sampling volume from which Raman photons are collected (the focal point is inside the bottle); this facilitates the generation of Raman photons within the collection volume, while suppressing the glass fluorescence from the outer surface [16]. However there has been little work on the detection of dissolved drugs in non-transparent plastic containers.

Here we dissolved acetaminophen (paracetamol) into methanol to create clear solutions with concentrations of 10, 15 and 20% (w/w). A white semi-opaque plastic bottle (1.5 mm wall thickness; HPDE plastic) was used to contain the solutions. Spot and ring spectra were obtained (30×2 second accumulations) and a scaled subtraction was performed, which was effective at removing the container bands. Figure 4a shows the SORS analysis for the 20% solution. The peaks due to acetaminophen can be identified, along with the prominent methanol peak at ~ 1030 cm^{-1}. Note that the wider peaks are a normal side effect for dissolved compounds, due to the greater freedom of the molecules in a solution compared to the solid form. Nonetheless, the peaks in the SORS spectrum correlate well with the peaks in the reference spectrum.

The characteristic peaks of the concealed drug would be sufficient to raise suspicion regarding the contents of this container [16]. However, it is also possible to suppress the methanol peak by subtracting a spectrum taken of pure methanol. This was conducted for each concentration and the results are shown in Figure 4b. There is a slight second-derivative shaped residual left over at ~ 1030 cm^{-1}, but the peaks for acetaminophen are clear in each test.

Effectively, this demonstration has succeeded despite 'double concealment' of this drug – both by dissolution in a transparent solvent and containment in a semi-opaque plastic container. It is expected that smugglers would probably use high concentrations so as to maximize the amount of drug concealed [16]. Nonetheless, the Raman spectrum was also successfully retrieved for lower concentrations of dissolved acetaminophen (10% and 15%, w/w), without significant deterioration in the signal quality (Figure 4b).

Postal packages and envelopes concealing white powders

A padded postbag was probed with the SORS device. The postbag had a thick white paper exterior (a few hundred microns thick) and an inner layer of transparent bubble wrap which are together a few millimeters in thickness. This type of postbag has a sturdy, rugged construction and is commonly used to post items that require a strong protective package. A sample of barium sulfate powder, a common filler in pharmaceutical preparations (~ 5 g in a small plastic bag, 1 cm depth), was placed inside and the laser was incident on the exterior of the postbag near the position of the concealed sample. Spot and ring measurements were taken with ten, 5-second exposures. The spot measurement yielded significant fluorescence originating from the paper (Figure 5).

The ring measurement was taken and the spectra were scaled to the envelope peak at ~1085 cm^{-1} (assumed calcium carbonate, a common whitener in paper) and a scaled subtraction was performed. The result closely matches the reference spectrum for barium sulfate (Figure 5) indicating successful identification of the concealed powder.

Matousek et al. have demonstrated interrogation of envelopes containing powders, including the identification of sugar within an envelope [9]. The researchers adopted a transmission geometry for this work because of the thinness of the sample. To confirm their results, we probed a thick yellow paper envelope containing a 1 mm-thick layer of acetaminophen using transmission Raman. First the empty envelope was probed, giving strong fluorescence emission from the paper (Figure 6). Such a spectrum would be expected from a legitimate letter containing only paper. Once the drug was placed inside, however, a clear acetaminophen spectrum could be obtained through the envelope using the transmission geometry (5 × 1 second accumulations) (Figure 6).

Recommendations and considerations for in-field SORS utilization

This work highlights some important practical considerations regarding SORS and its use in the field.

The above data show that the SORS technique is most effective with samples that are highly scattering and arranged in a thick layer. This is because the photons collected during the spatially offset measurement are generated deep within the sample [5]. Thus, it is advantageous to have sufficient underlying sample extending to a depth at least equal to the penetration depth of the laser light. In the case of SORS using NIR light, depending on the scattering, absorption and transmission characteristics of the sample and container, the penetration depth of the excitation light may be of the order of a few centimeters [5, 10], and thus having a layer of material of this thickness will enable the maximal Raman signals to be produced and collected. It is also expected that those samples that are well-known strong Raman scatterers in conventional Raman analysis will produce stronger signals in the SORS geometry as well. When the sample is thinner, the amount of material which may contribute to Raman scattering is less and there is greater transmission loss of the excitation light through the sample. This was noted initially when probing the envelope in the backscattered collection geometry (data not shown), where the signal collected from the thin layer of acetaminophen inside was relatively weak. In contrast, a thicker layer even allowed identification through more formidable barriers, such as a padded postbag and the opaque plastic bottle shown here, because the thicker sample provided for generation of more Raman photons. In the case of thinner samples the transmission geometry is expected to be beneficial, since locating the collection optics on the opposite side

would block the fluorescence and Raman emission from the illuminated side. Matousek et al. also adopted a transmission Raman geometry when detecting a concealed powder inside a paper envelope [9]. Therefore, it seems the transmission Raman analysis would be well suited to the routine scanning of very thin parcels and letters in mail screening and security applications.

SORS overlaps somewhat with the associated research field of 'wide-area illumination' (WAI) and its application for Raman spectroscopy [15, 18, 19]. For the analysis of thin samples, such as the envelope, the beam could also be expanded to increase sampling area, in a mode similar to WAI. This would gather information from a broader area of the sample and may thus increase the overall Raman signal. The notable difference between SORS and WAI is that WAI spectra cannot yield pure spectra of the concealed material, but include Raman bands (and fluorescence) from the container material [19]. This is because WAI involves the acquisition of only one Raman spectrum; the concept of a spatial offset to reduce the surface-originating signals and a subsequent spectral subtraction is not utilized and is the unique attribute of SORS. In practical situations, it would be possible to implement WAI in the cases where the container material was known or unchanging between different samples, or where the container makes a negligible contribution to the overall collected spectrum; thus, the applications of WAI are predominantly in production line settings where sample/container consistency is maintained, or where the samples are not inside packaging [15, 18, 19]. The notable strength of SORS, however, is that it requires no prior knowledge of the container material.

The SORS technique is very forgiving compared to, for example, confocal Raman which, although permitting the retrieval of Raman signals at shallow sub-surface depths and some rejection of surface Raman signals, requires careful placement of the focal plane inside the sample volume of interest, below the wall of the container or surface layer. SORS relies on a spatial offset rather than carefully positioned depth-resolved measurements. The alignment of the optics needs only to be accurate within a few millimeters, rather than to the level of tens or hundreds of microns, as with conventional backscattering and/or confocal techniques [9]. An example is the antibiotic capsule which, in confocal analysis, would require placement so that the focal plane was precisely within the capsule contents; in contrast, using SORS, the tolerance is much larger and the alignment of the capsule with the laser beam can be done quickly and by eye with sufficient accuracy. Moreover, SORS is able to probe depths extending well beyond those of confocal or other Raman techniques [5, 9], owing to the ability to achieve significant suppression of the surface Raman and fluorescence signals. Together, these attributes make SORS an attractive Raman geometry for field use [4, 9].

Although the discovery of SORS was a considerable accomplishment – thereby unlocking the ability to acquire pure, chemically specific spectra of substances behind barriers such as

polymers, paper and glass – there are notably some materials that remain difficult candidates for analysis with this technique, that are expected to be fundamental limitations of any approach based on Raman spectroscopy. Many black plastics (but not all) contain large amounts of carbon as the blackening pigment. Carbon is an efficient absorber of NIR light and is thus a formidable barrier for any mode of Raman analysis: analysis would require both the transmission of excitation light through the container wall, and reemergence of the wavelength-shifted Raman photons back through the wall, and this small signal would be difficult to measure. Similarly, any other strong absorbers of NIR light would be difficult targets. Various samples of thick grey cardboards, including corrugated cardboard, could not be penetrated in our tests (data not shown). It is also likely to be difficult to analyze drugs in cardboard packets, although the sealed blister pack itself could be analyzed. In some cases it might be possible to use longer-wavelength excitation light (e.g. ~ 1 micron or longer), although there exists the fundamental drawback of a lower Raman scattering efficiency (which decays as the fourth power of the excitation wavelength) [7] and the markedly diminished sensitivity of CCD detectors in this region, which would necessitate other detectors (e.g. InGaAs photodiode arrays) being used. Notably, water absorption also increases with longer wavelengths. Nonetheless, these characteristics are intrinsic within any Raman spectroscopic system and are not specific to SORS. Finally, metal containers are impenetrable to light and would be incompatible with SORS.

While this work has considered SORS analysis of drugs, it is notable that SORS has wide-ranging applicability for chemically specific identification of a broad range of items, including chemicals, explosives, food stuffs and biological agents, which are of obvious importance in forensics and national security settings [1, 9, 20-23]. There are also a range of emerging biomedical applications including the non-invasive analysis of bones through skin (ultimately aiming to detect bone diseases and degeneration) [11, 24-26] and the detection of breast cancer lesions [27-32]. Spatially offset Raman techniques are likely to remain a burgeoning and important research topic for many years ahead.

Conclusions

The results presented here show the utility of SORS for detecting various types of drugs and pharmaceuticals in a range of non-transparent containers. The results build on previous work, demonstrating new examples of successful SORS analysis, and raise various practical considerations for analyzing different types of containers and samples. The key advantages of SORS are its non-invasive and non-contact nature, relatively short analysis times, the capability of penetrating opaque (diffusely scattering) containers, and its ability to obtain chemically specific data to identify concealed substances. While there is no ‘silver bullet’ technique that

could detect drugs in every foreseeable mode of concealment, it is clear that SORS and related Raman spectroscopic techniques will find growing application in the customs and security applications alongside other techniques currently in use. SORS will also contribute to the technology used to scrutinize and inspect the production of tablets, capsules and other pharmaceutical products, and may help prevent the dangerous and increasing trend of pirated and counterfeit drugs being found within the supply chain.

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Authorship statement

The authors declare no conflicts of interest. WO and EI composed initial manuscript drafts. All authors contributed to manuscript reviews and were involved in devising and/or conducting the experiments reported herein.

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Figure captions

Figure 1. (a) Schematic diagram of the SORS [5] and inverse SORS (used here) [11, 12] geometries for probing samples. The former geometry involves illumination and collection from single points separated by a given spatial offset; the latter collects Raman photons from a point at the center of a surrounding ring of illumination. (b) Schematic diagram of the inverse SORS instrument design.

Figure 2. Raman spectrum for probing of phenylephrine hydrochloride inside an opaque plastic (HDPE) container with a 1 mm wall thickness (asterisks denote the dominant HDPE container peaks). The top two spectra are the raw data for the spot and ring measurements. The middle spectrum, provided for explanatory purposes, is the intermediate step of scaling the spectra so that the container peaks overlap; it is apparent that the ring measurement has collected a greater proportion of subsurface Raman photons relative to surface Raman photons. Subtraction of the scaled spectra (ring – spot) produces the SORS result, which closely matches the reference spectrum for phenylephrine hydrochloride (bottom). Acquisition times were 10 seconds for each spectrum.

Figure 3. Raman spectra of the antibiotic capsule inside a blister pack.

Figure 4. (a) Results for 20% (w/w) acetaminophen dissolved in methanol and concealed inside a semi-opaque plastic bottle with 1.5 mm wall thickness. The prominent peak at $\sim 1030\ cm^{-1}$ is from the methanol. (b) Spectra of acetaminophen in methanol at concentrations of 10, 15 and 20% (w/w), after subtracting the methanol spectrum from the SORS result. Note the widened peaks result from the dissolution of acetaminophen in a solvent.

Figure 5. Raman spectra of a padded postbag containing a sample of barium sulfate.

Figure 6. Raman spectra of a yellow envelope containing acetaminophen powder (here the transmission Raman geometry was used).

Figures

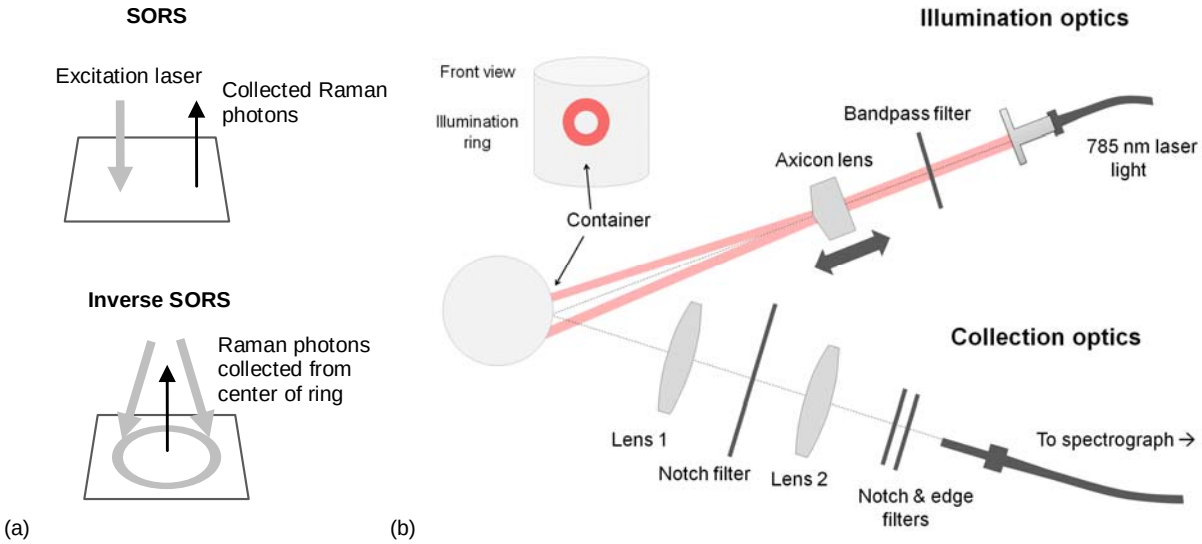


Figure 1.

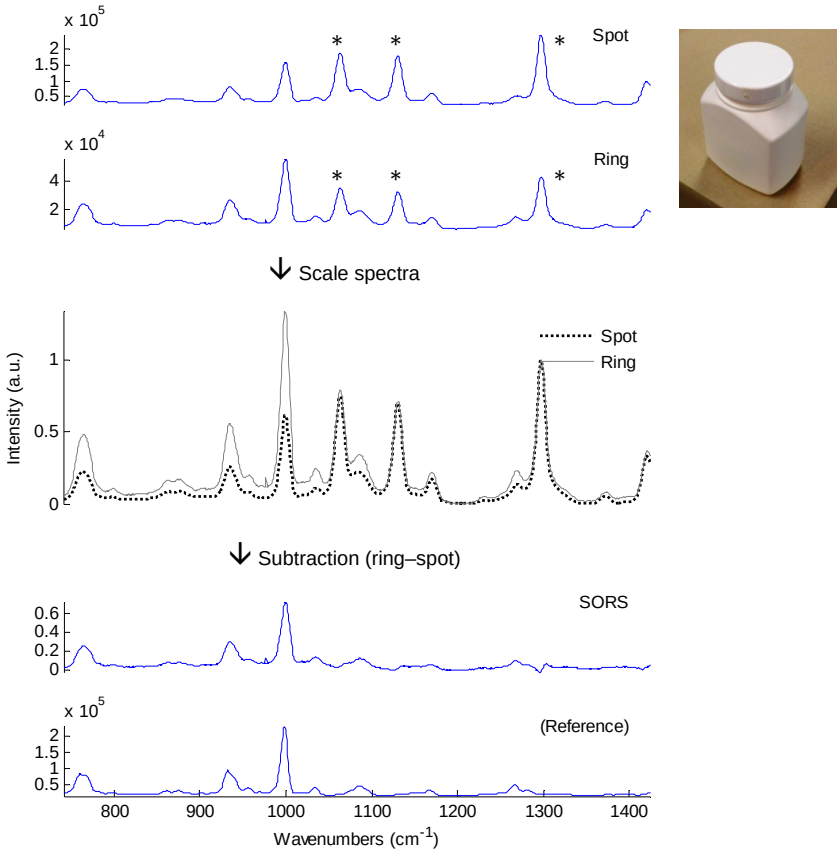


Figure 2.

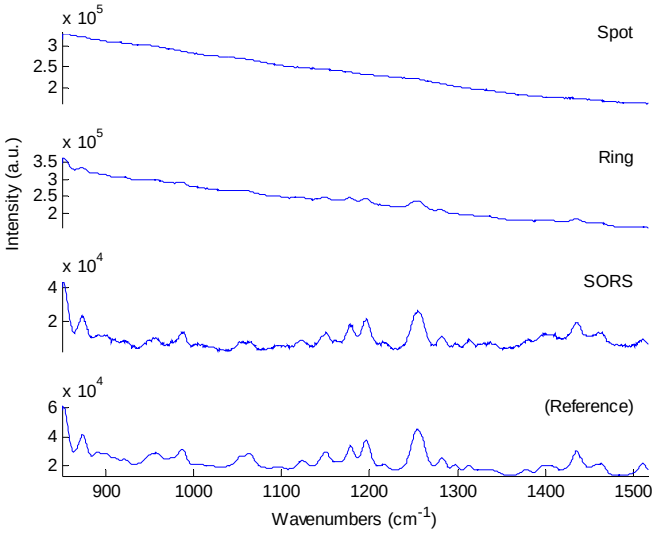
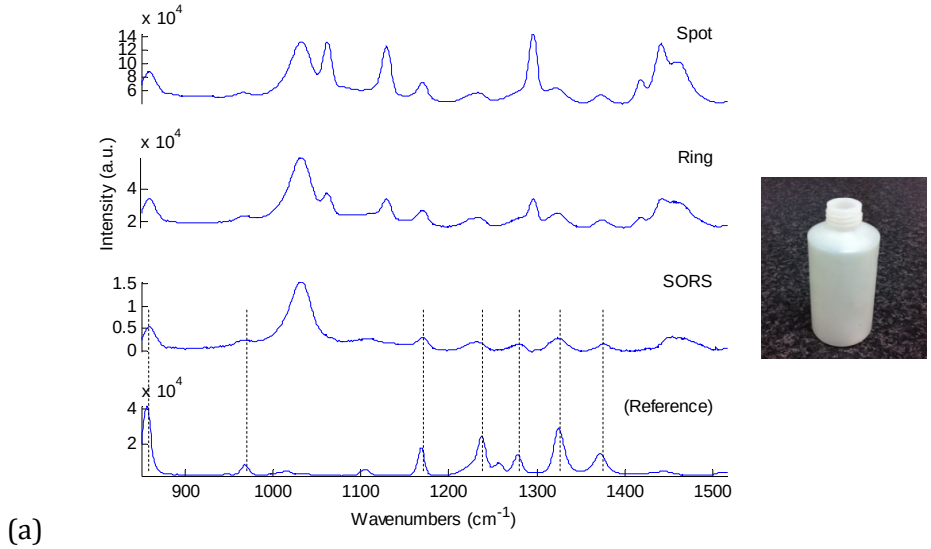
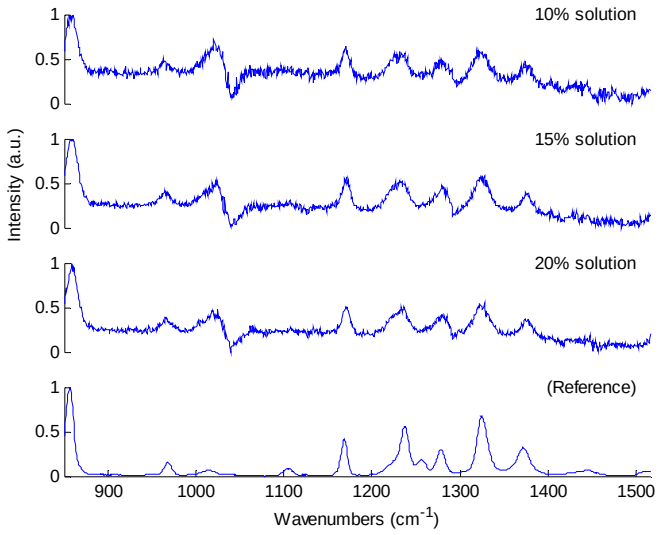

x 10^5
Spot
x 10^5
Ring
Intensity (a.u.)
x 10^4
SORS
x 10^4
(Reference)
900
1000
1100
1200
1300
1400
1500
Wavenumbers (cm^-1)

Figure 3.


x 10^4
Spot
Ring
SORS
(Reference)
Intensity (a.u.)
Wavenumbers (cm^-1)

(a)


10% solution
15% solution
20% solution
(Reference)
Intensity (a.u.)
Wavenumbers (cm^-1)

(b)

Figure 4.

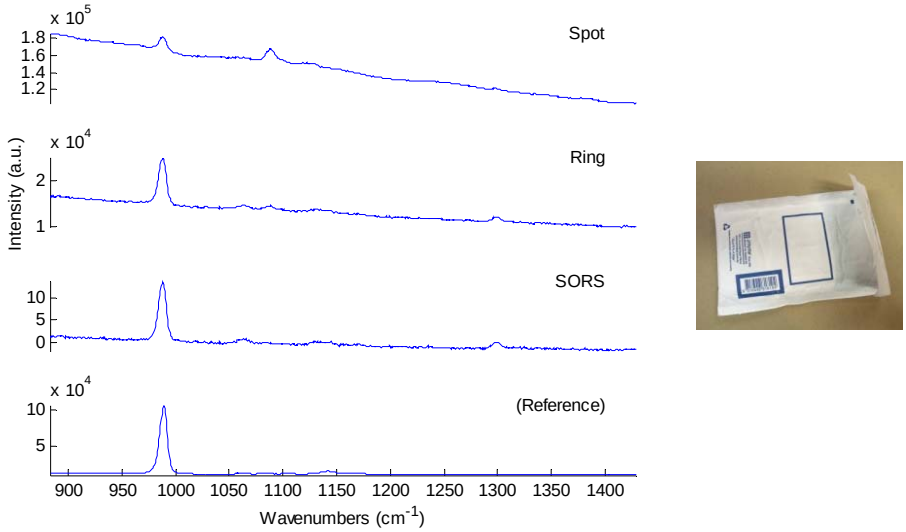


Figure 5.

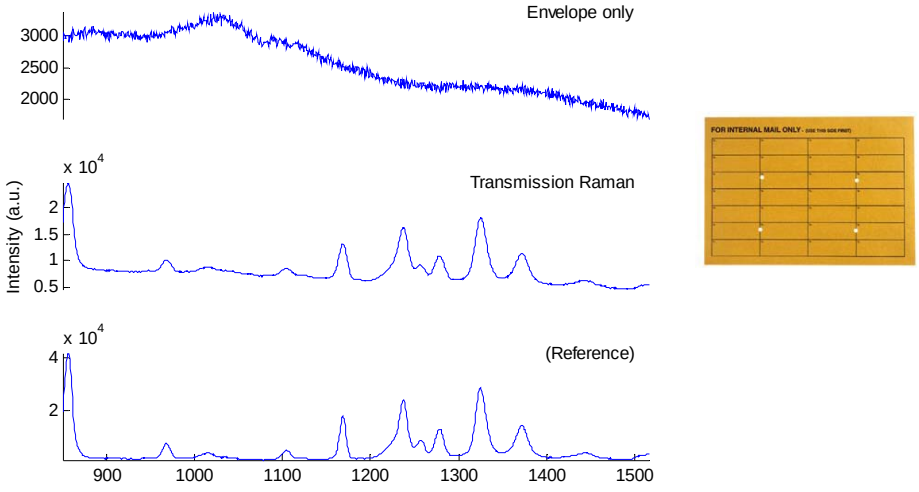


Figure 6.